PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 07 December 1999 (07.12.99)	
International application No. PCT/IB99/00740	**Applicant's or agent's file reference** 339869/18107
International filing date (day/month/year) 16 April 1999 (16.04.99)	**Priority date** (day/month/year) 16 April 1998 (16.04.98)
Applicant COLE, Stewart et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 November 1999 (08.11.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Juan Cruz

Telephone No.: (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 99/00740

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/70 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PHILIPP W.J. ET AL.,: "Physical mapping of mycobacterium bovis BCG pasteur reveals differences from the genome map of mycobacterium tuberculosis H37Rv and from M. bovis" MICROBIOLOGY, vol. 142, - 1996 pages 3135-3145, XP002118720 cited in the application the whole document --- -/--	1-50

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 1999	27/10/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Müller, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 99/00740

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	KIM U -J ET AL: "Construction and characterization of a human bacterial artificial chromosome library" GENOMICS, vol. 34, 1 June 1996 (1996-06-01), pages 213-218, XP002081197 ISSN: 0888-7543 cited in the application the whole document	1-50
A	WO 93 03187 A (AMOCO CORP) 18 February 1993 (1993-02-18) see whole doc. esp. claims	1-50
A	WO 93 18186 A (UNIV CALIFORNIA) 16 September 1993 (1993-09-16) see whole doc. esp. claims	1-50
P,X	BROSCH R. ET AL.,: "use of a mycobacterium tuberculosis H37Rv bacterial artificial chromosome library for genome mapping sequencing, and comparative genomics" INFECTION AND IMMUNITY, vol. 66, no. 5, - May 1998 (1998-05) pages 2221-2229, XP002104659 the whole document	1-50
P,A	COLE S T ET AL: "Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence" NATURE, vol. 393, 11 June 1998 (1998-06-11), pages 537-544, XP002087941 ISSN: 0028-0836 the whole document	1-50

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 99/00740

Patent document cited in search report	Publication date	Patent fan. member(s)	Publication date
WO 9303187 A	18-02-1993	EP 0554437 A	11-08-1993
		JP 6502082 T	10-03-1994
		US 5648481 A	15-07-1997
WO 9318186 A	16-09-1993	CA 2131543 A	16-09-1993
		EP 0631635 A	04-01-1995
		JP 7505053 T	08-06-1995
		US 5665549 A	09-09-1997
		US 5721098 A	24-02-1998
		US 5856097 A	05-01-1999

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PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
339869/18107		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IB 99/00740	16/04/1999	16/04/1998
Applicant		
INSTITUT PASTEUR et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 99/00740

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/70 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 1999	27/10/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Müller, F

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Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/IB 99/00740

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	KIM U -J ET AL: "Construction and characterization of a human bacterial artificial chromosome library" GENOMICS, vol. 34, 1 June 1996 (1996-06-01), pages 213-218, XP002081197 ISSN: 0888-7543 cited in the application the whole document	1-50
A	WO 93 03187 A (AMOCO CORP) 18 February 1993 (1993-02-18) see whole doc. esp. claims	1-50
A	WO 93 18186 A (UNIV CALIFORNIA) 16 September 1993 (1993-09-16) see whole doc. esp. claims	1-50
P,X	BROSCH R. ET AL.,: "use of a mycobacterium tuberculosis H37Rv bacterial artificial chromosome library for genome mapping sequencing, and comparative genomics" INFECTION AND IMMUNITY, vol. 66, no. 5, - May 1998 (1998-05) pages 2221-2229, XP002104659 the whole document	1-50
P,A	COLE S T ET AL: "Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence" NATURE, vol. 393, 11 June 1998 (1998-06-11), pages 537-544, XP002087941 ISSN: 0028-0836 the whole document	1-50

3

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 99/00740

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9303187 A	18-02-1993	EP 0554437 A	11-08-1993
		JP 6502082 T	10-03-1994
		US 5648481 A	15-07-1997
WO 9318186 A	16-09-1993	CA 2131543 A	16-09-1993
		EP 0631635 A	04-01-1995
		JP 7505053 T	08-06-1995
		US 5665549 A	09-09-1997
		US 5721098 A	24-02-1998
		US 5856097 A	05-01-1999

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
339869/18107		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB99/00740	16/04/1999	16/04/1998
International Patent Classification (IPC) or national classification and IPC		
C12N15/70		
Applicant		
INSTITUT PASTEUR et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 14 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/11/1999	13.07.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bretherick, J Telephone No. +49 89 2399 8415

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB99/00740

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-7,9-13,16,17,19-122, 124-131	as originally filed			
8,14,15,18,123	as received on	21/06/2000	with letter of	19/06/2000

Claims, No.:

1-53	as received on	21/06/2000	with letter of	19/06/2000

Drawings, sheets:

1/9-9/9	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

see separate sheet

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-7,9-27,29-53 (where this subject-matter does not refer to claim 8 or 28)
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-7,9-27,29-53 (where this subect-matter does not refer to claim 8 or 28)
Industrial applicability (IA)	Yes:	Claims	1-7,9-27,29-53 (where this subject-matter does not refer to claim 8 or 28)
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1. Regarding Part I:

a. The references to the deposition number I-2049 and the date of deposition of June 30 1998 inserted into the amended description pages 8, 14, 15, 18 and 123 and into newly amended claims 8 and 28 have not been taken into account during this examination. The requirements of R. 91.1 g)ii PCT have not been met since although it is clear that information in this respect has been omitted in error, it is not clear precisely what information should be included in order to rectify the error. Thus these pages and claims do not satisfy the requirements of Art. 19(2) PCT, since they include additional information.

2. Regarding Part VIII, Art. 6 PCT:

a. The designations of the BAC groups of claim 24 as Rv101 etc as recited in the claims are arbitrary and convey no meaning. This also applies to the embodiments of claim 25 and to the subject-matter of claim 30 with respect to the arbitrary designations used therein.

b. Claim 11 refers to a purified polynucleotide of interest that has been isolated according to the method of claim 9. Claim 11 is not characterised in terms of technical features **per se**, only by reference to the way in which it has been obtained and is therefore unclear, since no technical distinction can be implied with respect to the products derived from such methods. The above unclarity also applies, **mutatis mutandis** with respect to claims 12, 17, 18, 21 (for a pair of purified polynucleotides according to claim 11), 29, re. part (a), 31 re. parts (a) and (b), 34 re. part (a), 36 re. part (a), 37 re. parts (a) and (c), 38 re. part (a), 39 re. part (a), 40 re. part (a), 47 and 48.

The lack of clear characterisation of claim 11 moreover, implies a lack of novelty **per se** (see observations regarding Part V below).

c. There is no further definition, either functional or otherwise of the polynucleotide which hybridises under stringent conditions in part (c) of claim 14. This is therefore indeterminate. This also applies, **mutatis mutandis** to part (c) of claim 20.

d. The polynucleotide of claim 17 is also indeterminate, since the term "involved in the pathogenicity of a Mycobacterium strain" is itself an indeterminate functional attribute which cannot be used to precisely define the polynucleotide in question. Claim 18 is also not a precise characterisation. The structure of all or part of a "Polymorphism Glycine Rich Sequence" is not apparent from the claim.

e. Since there is no precise and unique art definition of what constitutes the size and structure of vectors termed "bacterial artificial chromosome vectors", which renders such vectors even without inserted DNA unique over others of the art, the subject-matter of claim 26 is not clear **per se**. The dependent claims 29 (re. claim 26), 30, 36, 37, 39, 40, 41, 42, 49, 50 and 51 are thus also unclear.

2. Regarding Part V, Art. 33 PCT:

a. The subject-matter of the claims subject to examination are considered to enjoy the priority right of application US 09/060,756, filed 06/04/1998.

Consequently, Brosch et al. (1998), Infection and Immunity, Vol. 66 pp. 2221-2229 and also Cole et al. (1998) Nature Vol. 393 pp. 537-544 are currently not considered to be art for the purposes of Art. 33 PCT.

b. Philipp et al. (1996) Microbiology Vol. 142 pp. 3135-3145 reports the physical mapping of differences between the genome maps, M. bovis BCG and M. tuberculosis H37Rv which also include loci for the protein antigens ESAT-6 and mpt64. The maps and comparisons were made using material, **inter alia** from genomic cosmid DNA libraries (see Summary and Methods). The difference between the subject-matter of claim 26 differs from the art in the use of bacterial artificial chromosome vectors for the make up of the DNA library.

c. Since BACs have been used as vectors for the creation of a number of genomic DNA libraries (for examples (see the art cited in the passage of current description page 2, lines 10-12, which includes Kim et al. (1996) Genomics Vol. 34. pp. 213-218)), the use of same to make genomic libraries of **Mycobacterium** strains and species would be obvious, in view of their advantages in general over cosmids. There would furthermore, not appear to be any surprising effects and/or

advantages associated with such an implementation in the context of Mycobacterium. Generic claim 26 thus lacks an inventive step under Art. 33(1)(3) PCT.

The same applies **mutatis mutandis** with respect to the specific subject-matter of claims dependent on claim 26, since the skilled person would consider the use of BACs as the basis for **any** genomic DNA library based on **Mycobacterium** to be a logical option. The same also applies with respect to the subject-matter of claims 22-25.

d. Claim 1 is directed to a method for isolating a polynucleotide of interest that is present in the genome of a mycobacterium strain and/or is expressed by said mycobacterium strain and is absent or altered in the genome of a different mycobacterium strain and/or is not expressed in said different mycobacterium strain, said method comprising the use of at least one clone belonging to a genomic DNA library of a given **Mycobacterium** strain, said DNA library being cloned in a bacterial artificial chromosome (BAC) vector.

This and the remaining claimed subject-matter is the an extension application of comparative genomic studies using BAC vector based genomic libraries and the use of theses in order to identify polynucleotides and corresponding encoded polypeptides in various known strains and spp. of **Mycobacterium**. The principle of this technique has been applied in other fields, for example in the use of BACs for the cloning of human genomic libraries. The identification of differing loci has already been achieved in Philipp et al. (1996) supra. The skilled person would consider the identification and isolation of polynucleotides and thereby encoded polypeptides to be a logical extension of the prior art.

been deposited in the Collection Nationale de Cultures de Microorganismes (CNCM) on June 30, 1998 under the accession number I-2049.

Thus, as a specific embodiment of the above described method for isolating a polynucleotide of interest said method makes use of at least one BAC-based DNA library that has beeen constructed from the genomic DNA of *Mycobacterium tuberculosis*, more specifically of the H37Rv strain and particularly of the DNA library deposited in the accession number I-1945.

In another specific embodiment of the above described method for isolating a polynucleotide of interest said method makes use of at least one BAC-based DNA library has beeen constructed from the genomic DNA of *Mycobacterium bovis* BCG, more specifically of the Pasteur strain and particularly of the DNA library deposited in the accession number I-2049.

In more details, the method according to the invention for isolating a polynucleotide of interest may comprise the following steps :

a) isolating at least one polynucleotide contained in a clone of a BAC-based DNA library of mycobacterial origin;

b) isolating :

- at least one genomic or cDNA polynucleotide from a mycobacterium, said mycobacterium belonging to a strain different from the strain used to construct the BAC-based DNA library of step a); or alternatively
- at least one polynucleotide contained in a clone of a BAC-based DNA library prepared from the genome of a mycobacterium that is different from the mycobacterium used to construct the BAC-based DNA library of step a);

c) hybridizing the at least one polynucleotide of step a) to the at least one polynucleotide of step b);

d) selecting the at least one polynucleotide of step a) that has not formed a hybrid complex with the at least one polynucleotide of step b);

e) characterizing the selected polynucleotide.

Following the above procedure, the at least one polynucleotide of step a) may be prepared as follows :

1) digesting at least one recombinant BAC clone by an appropriate resctriction endonuclease in order to isolate the polynucleotide insert of interest from the vector genetic material;

2) optionally amplifying the resulting polynucleotide insert;

disease and elicits a variable antibody response suggesting either that individuals mount different immune responses or that this PGRS-protein may not be produced in this form by all strains of *M. tuberculosis*. In other words, at least some PE_PGRS coding sequences encode for proteins that are involved in the recognition of *M. tuberculosis* by the immune system of the infected host. Therefore, differences in the PGRS sequences could represent the principal source of antigenic variation in the otherwise genetically and antigenically homogeneous bacterium.

By performing the method of the invention using the *M. tuberculosis* BAC based DNA library I-1945, the inventors have discovered the occurence of sequence differences between a given PGRS encoding ORF (ORF reference on the genomic sequence of *M. tuberculosis* Rv0746) of *M. tuberculosis* and its counterpart sequence in the genome of *M. bovis* BCG.

More precisely, the inventors have determined that one ORF contained in BAC vector N° Rv418 of the *M. tuberculosis* BCG I-1945 DNA library carries both base additions and base deletions when compared with the corresponding ORF in the genome of *M. bovis* BCG that is contained in the BAC vector N° X0175 of the *M. bovis* BCG I-2049 DNA libary. The variations observed in the base sequences correspond to variations in the C-terminal part of the aminoacid sequence of the PGRS ORF translation product.

As shown in Figure 6, an amino acid stretch of 9 residues in length is present in this *M. tuberculosis* PGRS (ORf reference Rv0746) and is absent from the ORF counterpart of *M. bovis* BCG, namely the following amino acid sequence:

NH_2-GGAGGAGGSSAGGGGAGGAGGAGGWLLGD-COOH.

Furthermore, Figure 6 shows also that an amino acid stretch of 45 residues in length is absent from this *M. tuberculosis* PGRS and is present in the ORF counterpart of *M. bovis* BCG, namely following amino acid sequence:

NH_2-GAGGIGGIGGNANGGAGGNGGTGGQLWGSGGAGVEGGAAL SVGDT-COOH.

Similar observations were made with PPE ORF Rv0442, which showed a 5 codon deletion relative to a *M. bovis* amino acid sequence.

Given that the polymorphism associated with the PE-PGRS or PEE ORFS resulted in extensive antigenic variability or reduced antigen presentation, this would be of immense significance for vaccine design, for understanding

protective immunity in tuberculosis and, possibly, explain the varied responses seen in different BCG vaccination programmes.

There are several striking parallels between the PGRS proteins and the Epstein-Barr virus-encoded nuclear antigens (EBNA). Both polypeptide families are glycine-rich, contain Gly-Ala repeats that represent more than one third of the molecule, and display variation in the length of the repeat region between different isolates. The Gly-Ala repeat region of EBNA1 has been shown to function as a *cis*-acting inhibitor of antigen processing and MHC class I-restricted antigen presentation (Levitskaya et al., 1995). The fact that MHC class I knock-out mice are extremely suscepible to *M. tuberculosis* underlines the importance of MHC class I antigen presentation in protection against tuberculosis. Therefore, it is possible that the PE/PPE protein family also play some role in inhibiting antigen presentation, allowing the bacillus to hide from the host's immune system.

As such the novel and nonobvious PGRS polynucleotide from *M. bovis* which is homolog to the *M. tuberculosis* ORF Rv0746, and which is contained in the BAC clone N° X0175 (See Table 4 for SP6 and T7 end-sequences of clone n° X0175) of the I-2049 *M. bovis* BCG BAC DNA library is part of the present invention, as it represents a starting material in order to define specific probes or primers useful for detection of antigenic variability in mycobacterial strains, possible inhibition of antigen processing as well as to differentiate *M. tuberculosis* from *M. bovis* BCG.

Thus, a further object of the invention consists in a polynucleotide comprising the sequence SEQ ID N°4.

Polynucleotides of interest have been defined by the inventors as useful detection tools in order to differentiate *M. tuberculosis* from *M. bovis* BCG. Such polynucleotides are contained in the 45 aminoacid length coding sequence that is present in *M. bovis BCG* but absent from *M. tuberculosis*. This polynucleotide has a sequence beginning (5'end) at the nucleotide at position nt 729 of the sequence SEQ ID N°4 and ending (3'end) at the nucleotide in position nt 863 of the sequence SEQ ID N°4.

Thus, part of the present invention is also a polynucleotide which is chosen among the following group of polynucleotides :

a) a polynucleotide comprising at least 8 consecutive nucleotides of the nucleotide sequence SEQ ID N°5 ;

Rv106; Rv39; Rv255; Rv74; Rv355; Rv268; Rv58; Rv173; Rv264; Rv417; Rv401; Rv144; Rv302; Rv81; Rv163; Rv281; Rv221; Rv420; Rv175; Rv86; Rv412; Rv73; Rv269; Rv214; Rv287; Rv42; Rv143.

The polynucleotides disclosed in Table 3 may be used as probes in order to select a given clone of the BAC DNA library I-1945 for further use.

The invention also provides for a BAC-based *Mycobacterium bovis* strain Pasteur genomic DNA library that has been deposited in the Collection Nationale de Cultures de Microorganismes on June 30, 1998 under the accession number I-2049.

A further object of the invention consists in a recombinant BAC vector which is chosen among the group consisting of the recombinant BAC vectors belonging to the BAC-based DNA library I-2049. This DNA library contains approximately 1600 clones. The average insert size is estimated to be ~80 kb.

Generally, a recombinant BAC vector of interest may be chosen among the following set or group of BAC vectors contained in the BAC-based DNA library I-2049 :

X0001; X0002; X0003; X0004; X0006; X0007; X0008; X0009; X0010; X0012; X0013; X0014; X0015; X0016; X0017; X0018; X0019; X0020; X0021; X0175.

The end sequences of the polynucleotide inserts of each of the above clones corresponding respectively to the sequences adjacent to the T7 promoter and to the Sp6 promoter on the BAC vector are shown in Table 4.

The polynucleotides disclosed in Table 4 may be used as probes in order to select a given clone of the BAC DNA library I-2049 for further use.

Are also part of the invention the polynucleotide inserts that are contained in the above described BAC vectors, that are useful as primers or probes.

These polynucleotides and nucleic acid fragments may be used as primers for use in amplification reactions, or as nucleic probes.

PCR is described in the US patent N° 4,683,202. The amplified fragments may be identified by an agarose or a polyacrylamide gel electrophoresis, or by a capillary electrophoresis or alternatively by a chromatography technique (gel filtration, hydrophobic chromatography or ion exchange chromatography). The specificity of the amplification may be ensured by a molecular hybridization using, for example, one of the initial primers as nucleic probes.

Amplified nucleotide fragments are used as probes in hybridization reactions in order to detect the presence of one polynucleotide according to the

Table 4 : End-sequences of the polynucleotide inserts cloned in the named recombinant BAC vectors contained in the I-2049 *M. bovis* strain Pasteur genomic DNA library.

RvXXXSP6 corresponds to the SP6 end-sequence of the clone RvXXX.

RvXXXT7 corresponds to the T7 end-sequence of the clone RvXXX.

RvXXXIS 1081 corresponds to a region located close to a copy of the IS1081 repetitive sequence (Insertion element).

The character « - » denotes an uncertain base residue.

Clone X0001

::::::::::::::::X0001SP6.seq::::::::::::::

AAG-
TCGGGTTTCCACACGCGCGGTTTGACCCTAGTCATATGTAATCATGTGTACCATGTGCGGGCGCTTTTCGACGGCCG
CGAACCACCGGA-ATTTCCTGTGATTTCACTGCATGCGTACCATCTGGCACAATTGAGCA-TTGTCT-
TCGCGGTGGTCGG-CGGGTTGCGTGCCGCCTGCTGCGA-ATGCACCA-
TAAGCCCGAACCCACCGGCTTGGTGACCACCGCACGCTGCGTGTGGGGGGTAACCACTCCGCGACCCCAAGGATGGT
CATTTCCAATGAACCGGCTGGACTTCGTCCA-A

::::::::::::::X0001T7.seq::::::::::::::

GTCGCGGTTCGATCGACCCGATCTTCACCTCGTAACCTCGATGCTTAGCAGGATCCAGCTTGACCGCGTTTGGCTCT
ACCCACTCTTTGAGTGGCGCCGTCGCCTGTGCCCCATCGGTGTTCATGACGAACGCTTCGAAAGACTTCCTCTTGTG
AGCCGGAATGTCTGCGTAAAGAAGTTCCATGTCCGGGAAGTAGACCCGGTCGCCCTCCACGTGGTACTCCTTCGAGG
TCCGCTTCTCGCCGGATCCGATAAACACCGGCCCCAGGCACCGCAGCGTGAGTTCGAACGGCTTCAGGTAGGTGTTC
ATGCGGCGGACTCCGGGAGTGCGAGAAATAGCGGTCGCGCGTAGCTGTAGACCGGATGGTTTCCGCCCAGGCTGACG
TCGAAGATGCCTCCTTGGAAGGGGCGCGA

Clone X0002

::::::::::::::X0002SP6.seq::::::::::::::

AACTCAAGTTTTTACGGTGATCGCGCATCACCTGGTTCATGAACTGGAAGCAGCGCAGCGCTTCCTTTTCGGCCGCA
ACATGAGCCAGCCTCTCGTCGGCGGTCGGGTGCAGGTGCTCGGGCAGCTCGGCCGCGACAGCCGCCTGACCCTGAAA
CCAGCTTCCATATCCCGCGAC-
AACGACGCCAGTCCGCTACGTAACCCCTCCGCGACTGTCCATGGACAACAGCGCGTTCTCCACCGACCGGGCCCGGG
TGT

::::::::::::::X0002T7.seq::::::::::::::

GTGCAGGTTTCGACAATGTGGTGCCGGTTCGGCGGCTACGTGCCATCGAGACACTGGCGCA-GCTATCGCACCCGTT
ATCGGCTGCGAGCAAATCGCGGTATGCGTTCTTGAGCATGAGTCGGCGACCGTCGTCATGGTCGACACCCACGACGG
AAAGACGCAGATCGCCGTCAAGCATGTGTGCCGCGGATTATCAGGACTGACCTCCTGGCTGACCGGCATGTTTGGTC
GCGATGCCTGGCGCCCGGCCGGCGTGGTCGTGGTCGGCTCGGATAGCGAGGTCAGCGAATTCTCGTGGCAGCTCGAA
AGGGTCCTGCCGGTGCCGGT

Clone X0003

::::::::::::::X0003SP6.seq::::::::::::::

TTCGAGTCATGCGCCCGCCTCGACCACGAA-ATGCACGTCG-
GGTTCGATCGACCCGATCTTCACCTCGTAACCTCGATGCTTAGCAGGATCCAGCTTGACCGCGTTTGGCTCTACCCA
CTCTTTGAGTGGCGCCGTCGCCTGTGCCCCATCGGTGTTCATGACGAACGCTTCGAAAGACTTCCTCTTGTGAGCCG
GAATGTCTGCGTAAAGAAGTTCCATGTCCGGGAAGTAGACCCGGTCGCCCTCCACGTGGTACTCCTTCGAGGTCCGC
TTCTC

CLAIMS

1. A method for isolating a polynucleotide of interest that is present in the genome of a mycobacterium strain and/or is expressed by said mycobacterium strain and that is absent or altered in the genome of a different mycobacterium strain and/or is not expressed in said different mycobacterium strain, said method comprising the use of at least one clone belonging to a genomic DNA library of a given mycobaterium strain, said DNA library being cloned in a bacterial artificial chromosome (BAC) vector.

2. The method according to claim 1, wherein the BAC-based DNA library has beeen constructed from genomic DNA of *Mycobacterium tuberculosis*.

3. The method according to claim 2, wherein the BAC-based DNA library has beeen constructed from genomic DNA of *Mycobacterium tuberculosis* strain H37Rv.

4. The method according to claim 3, wherein the BAC-based DNA library has been deposited in the Collection Nationale de Cultures de Microorganismes (CNCM) on November 19, 1997 under the accession number I-1945.

5. The method according to claim 1, wherein the BAC-based DNA library has been constructed from genomic DNA of *Mycobacterium bovis*.

6. The method according to claim 5, wherein the BAC-based DNA library has been constructed from the genomic DNA of *Mycobacterium bovis* BCG strain Pasteur.

7. The method according to claim 6, wherein said DNA library contains approximatively 1600 clones and wherein the genomic DNA is cloned into a recombinant pBeloBAC11 vector with an average insert size of approximately 80 kb.

8. The method according to claim 6 or 7, wherein the at least one BAC-based DNA library has been deposited in the Collection Nationale de Cultures de Microorganismes (CNCM) on June 30, 1998 under the accession number I-2049.

9. A method of isolating a polynucleotide of interest that is present in a genome of a first mycobacterium strain or that is expressed by the first mycobacterium strain and that is absent or altered in a genome of a second mycobacterium strain or that is not expressed by the second mycobacterium strain, said method comprising :

a) providing at least one polynucleotide contained in a clone of a bacterial artificial chromosome (BAC) DNA library of the first mycobacterium strain;

b) providing at least one genomic or cDNA polynucleotide from a second mycobacterium strain that is different from the first mycobacterium strain or at least one polynucleotide contained in a clone of a BAC DNA library prepared from the genome of the second mycobacterium strain;

c) contacting under hybridizing conditions the polynucleotide of step a) with the polynucleotide of step b); and

d) isolating the polynucleotide of step a) that has not formed a hybrid complex with the polynucleotide of step b).

10. The method of claim 9, wherein the polynucleotide contained in a clone of a BAC DNA library of the first or second mycobacterium strain is prepared by the following procedure :

1) digesting at least one recombinant BAC clone by an appropriate restriction endonuclease to yield a polynucleotide insert of interest; and

2) isolating the polynucleotide insert of interest.

11. A purified polynucleotide of interest that has been isolated according to the method of claim 9.

12. The purified polynucleotide of claim 11 which contains at least one Open Reading Frame (ORF).

13. The purified polynucleotide of claim 12, which is SEQ ID N0:1.

14. The purified polynucleotide of claim 12, wherein said polynucleotide is selected from the group consisting of :

a) a polynucleotide comprising at least 8 consecutive nucleotides of SEQ ID N0:1;

b) a polynucleotide having a sequence fully complementary to SEQ ID N0:1; and

c) a polynucleotide that hybridizes under stringent hybridization conditions with the polynucleotide defined in a) or with the polynucleotide defined in b).

15. The purified polynucleotide of claim 14, which is SEQ ID N0:2.

16. The purified polynucleotide of claim 14, which is SEQ ID N0:3.

17. The purified polynucleotide of claim 12, wherein the ORF encodes all or part of a polypeptide involved in the pathogenicity of a mycobacterium strain.

18. The purified polynucleotide of claim 12, wherein the ORF encodes all or part of a Polymorphism Glycine Rich Sequence (PGRS).

19. The purified polynucleotide of claim 18, which is SEQ ID N0:4.

20. The purified polynucleotide of claim 18, which is selected from the group consisting of:

a) a polynucleotide comprising at least 8 consecutive nucleotides the of SEQ ID N0:5 ;

b) a polynucleotide having a sequence that is fully complementary to SEQ ID N0:5 ;

c) a polynucleotide that hybridizes under stringent hybridization conditions with the polynucleotide defined in a) or with the polynucleotide defined in b).

21. A pair of the purified polynucleotides as claimed in claim 11.

22. A *Mycobacterium tuberculosis* strain Rv37 genomic DNA library that has been deposited in the Collection Nationale de Cultures de Microorganismes under accession number I-1945, wherein said genomic DNA library comprises recombinant bacterial artificial chromosome vectors.

23. A recombinant bacterial artificial chromosome (BAC) vector, which belongs to the genomic DNA library of claim 22.

24. The recombinant BAC vector of claim 23, which is selected from the group consisting of :

Rv101; Rv102; Rv103; Rv104; Rv105; Rv106; Rv107; Rv108; Rv109; Rv10; Rv110; Rv111; Rv112; Rv113; Rv114; Rv115; Rv116; Rv117; Rv118; Rv119; Rv11; Rv120; Rv121; Rv122; Rv123; Rv124; Rv126; Rv127; Rv128; Rv129; Rv130; Rv132; Rv134; Rv135; Rv136; Rv137; Rv138; Rv139; Rv13; Rv140; Rv141; Rv142; Rv143; Rv144; Rv145; Rv146; Rv147; Rv148; Rv149; Rv14; Rv150; Rv151; Rv152; Rv153; Rv154; Rv155; Rv156; Rv157; Rv159; Rv15; Rv160; Rv161; Rv162; Rv163; Rv164; Rv165; Rv166; Rv167; Rv169; Rv16; Rv170; Rv171; Rv172; Rv173; Rv174; Rv175; Rv176; Rv177; Rv178; Rv179; Rv17; Rv180; Rv181; Rv182; Rv183; Rv184; Rv185; Rv186; Rv187; Rv188; Rv18; Rv190; Rv191; Rv192; Rv193; Rv194; Rv195; Rv196; Rv19; Rv1; Rv201; Rv204; Rv205; Rv207; Rv209; Rv20; Rv214; Rv215; Rv217; Rv218; Rv219; Rv21; Rv220; Rv221; Rv222; Rv223; Rv224; Rv225; Rv226; Rv227; Rv228; Rv229; Rv22; Rv230; Rv231; Rv232; Rv233; Rv234; Rv235; Rv237; Rv240; Rv241; Rv243; Rv244; Rv245; Rv246; Rv247; Rv249; Rv24; Rv251; Rv252; Rv253; Rv254; Rv255; Rv257; Rv258; Rv259; Rv25; Rv260; Rv261; Rv262; Rv263; Rv264; Rv265; Rv266; Rv267; Rv268; Rv269; Rv26; Rv270; Rv271; Rv272; Rv273; Rv274; Rv275; Rv276; Rv277; Rv278; Rv279; Rv27; Rv280; Rv281; Rv282; Rv283; Rv284; Rv285; Rv286; Rv287; Rv288; Rv289; Rv28; Rv290; Rv291; Rv292; Rv293; Rv294; Rv295; Rv296; Rv29; Rv2; Rv301; Rv302; Rv303; Rv304; Rv306; Rv307; Rv308; Rv309; Rv30; Rv310; Rv311; Rv312; Rv313; Rv314; Rv315; Rv316; Rv317; Rv318; Rv319; Rv31; Rv32; Rv322; Rv327; Rv328; Rv329; Rv32; Rv330; Rv331; Rv333; Rv334; Rv335; Rv336; Rv337; Rv338; Rv339; Rv33; Rv340; Rv341; Rv343; Rv344; Rv346; Rv347; Rv348; Rv349; Rv34; Rv350; Rv351; Rv352; Rv353; Rv354; Rv355; Rv356; Rv357; Rv358; Rv359; Rv35; Rv360; Rv361; Rv363; Rv364; Rv365; Rv366; Rv367; Rv368; Rv369; Rv36; Rv370; Rv371; Rv373; Rv374; Rv375; Rv376; Rv377; Rv378; Rv379; Rv37; Rv381; Rv382; Rv383; Rv384; Rv385; Rv386; Rv387; Rv388; Rv389; Rv38; Rv390; Rv391; Rv392; Rv393; Rv396; Rv39; Rv3; Rv40; Rv412; Rv413; Rv414; Rv415; Rv416; Rv417; Rv418; Rv419;

Rv41; Rv42; Rv43; Rv44; Rv45; Rv46; Rv47; Rv48; Rv49; Rv4; Rv50; Rv51; Rv52; Rv53; Rv54; Rv55; Rv56; Rv57; Rv58; Rv59; Rv5; Rv60; Rv61; Rv62; Rv63; Rv64; Rv65; Rv66; Rv67; Rv68; Rv69; Rv6; Rv70; Rv71; Rv72; Rv73; Rv74; Rv75; Rv76; Rv77; Rv78; Rv79; Rv7; Rv80; Rv81; Rv82; Rv83; Rv84; Rv85; Rv86; Rv87; Rv88; Rv89; Rv8; Rv90; Rv91; Rv92; Rv94; Rv95; Rv96 and Rv9.

25. The recombinant BAC vector of claim 23, which is selected from the group consisting of:

Rv234; Rv351; Rv166; Rv35; Rv415; Rv404; Rv209; Rv272; Rv30; Rv228; Rv233; Rb38; Rv280; Rv177; Rv48; Rv374; Rv151; Rv238; Rv156; Rv92; Rv3; Rv403; Rv322; Rv243; Rv330; Rv285; Rv233; Rv219; Rv416; Rv67; Rv222; Rv149; Rv279; Rv87; Rv273; Rv266; Rv25; Rv136; Rv414; Rv13; Rv289; Rv60; Rv104; Rv5; Rv165; Rv215; Rv329; Rv240; Rv19; Rv74; Rv411; Rv167; Rv56; Rv80; Rv164; Rv59; Rv313; Rv265; Rv308; Rv220; Rv258; Rv339; Rv121; Rv419; Rv418; Rv45; Rv217; Rv134; Rv17; Rv103; Rv21; Rv22; Rv2; Rv270; Rv267; Rv174; Rv257; Rv44; Rv71; Rv7; Rv27; Rv191; Rv230; Rv128; Rv407; Rv106; Rv39; Rv255; Rv74; Rv355; Rv268; Rv58; Rv173; Rv264; Rv417; Rv401; Rv144; Rv302; Rv81; Rv163; Rv281; Rv221; Rv420; Rv175; Rv86; Rv412; Rv73; Rv269; Rv214; Rv287; Rv42 and Rv143.

26. A *Mycobacterium bovis* BCG strain Pasteur genomic DNA library, wherein said genomic DNA library comprises recombinant bacterial artificial chromosome vectors.

27. A *Mycobacterium bovis* BCG strain Pasteur genomic DNA library according to claim 26, wherein said DNA library contains approximatively 1600 clones and wherein the genomic DNA is cloned into a recombinant pBeloBAC11 vector with an average insert size of approximately 80 kb.

28. A *Mycobacterium bovis* BCG strain Pasteur genomic DNA library according to claim 26, that has been deposited in the Collection Nationale de

Cultures de Microorganismes (CNCM) on June 30, 1998 under the accession number I-2049.

29. A recombinant bacterial artificial chromosome (BAC) vector, which belongs to the genomic DNA library of claims 26 to 28.

30. A recombinant BAC vector according to claim 29, which is selected from the group consisting of:
X0001; X0002; X0003; X0004; X0006; X0007; X0008; X0009; X0010; X0012; X0013; X0014; X0015; X0016; X0017; X0018; X0019; X0020; X0021 and X0175.

31. A method for detecting a mycobacterial nucleic acid in a biological sample comprising the steps of:

a) contacting the recombinant BAC vector according to claim 23 or 29, or a purified polynucleotide according to claim 11 with the mycobacterial nucleic acid in the biological sample ; and

b) detecting a hybrid nucleic acid molecule formed between said recombinant BAC vector or said purified polynucleotide and the mycobacterial nucleic acid in the biological sample.

32. The method of claim 31, further comprising before step a), making the mycobacterial nucleic acid in the biological sample available to a hybridization reaction.

33. A method for detecting mycobacterial nucleic acid in a biological sample comprising the steps of:

a) contacting a first polynucleotide according to claim 11 that has been immobilized onto a substrate with the mycobacterial nucleic acid in the biological sample ; and

b) contacting a hybrid nucleic acid molecule formed between said first polynucleotide and the mycobacterial nucleic acid in the biological sample with a second, labeled polynucleotide according to claim 11, wherein said

second polynucleotide and said first polynucleotide have non-overlapping sequences.

34. The method of claim 33, further comprising before step a), making the mycobacterial nucleic acid in the biological sample available to a hybridization reaction.

35. The method of claim 33 or 34, further comprising before step b), removing the mycobacterial nucleic acid that is not hybridized with the immobilized first polynucleotide.

36. A method for detecting mycobacterial nucleic acid in a biological sample comprising the steps of:

a) contacting the mycobacterial nucleic acid in the biological sample with a pair of purified polynucleotides according to claim 21 ;

b) amplifying said mycobacterial nucleic acid ; and

c) detecting the amplified mycobacterial nucleic acid.

37. The method of claim 36, further comprising before step a), making the mycobacterial nucleic acid in the biological sample available to a hybridization reaction.

38. A kit for detecting a mycobacterium in a biological sample comprising:

a) a recombinant BAC vector according to claim 23 or 29, or a purified polynucleotide according to claim 11 ; and

b) reagents necessary to perform a nucleic acid hybridization reaction.

39. A kit for detecting a mycobacterium in a biological sample comprising:

a) a recombinant BAC vector according to claim 23 or 29, or a first polynucleotide according to claim 11 that is immobilized onto a substrate ;

b) reagents necessary to perform a nucleic acid hybridization reaction ; and

c) a second polynucleotide according to claim 11, wherein said second polynucleotide is radioactively or non-radioactively labeled, and wherein said second polynucleotide and said first polynucleotide have non-overlapping sequences.

40. A kit for detecting a mycobacterium in a biological sample comprising:

a) a pair of purified polynucleotides according to claim 20 ; and

b) reagents necessary to perform a nucleic acid amplification reaction.

41. A method for detecting the presence of a genomic DNA, a cDNA or a mRNA of a mycobacterium in a biological sample, comprising the steps of:

a) contacting the biological sample with a plurality of BAC vectors according to claim 23 or 29, or purified polynucleotides according to claim 11 that are immobilized on a substrate ; and

b) detecting the hybrid complexes formed.

42. A kit for detecting a genomic DNA, a cDNA or a mRNA of a mycobacterium in a biological sample, comprising:

a) a substrate on which a plurality of BAC vectors according to claim 23 or 29, or purified polynucleotides according to claim 11 have been immobilized.

43. A method for detecting a polynucleotide of mycobacterial origin in a biological sample, said method comprising:

a) aligning at least one polynucleotide contained in a recombinant BAC vector according to claim 23 or 29 on the surface of a substrate ;

b) contacting the polynucleotide in the biological sample with the substrate on which the polynucleotide of step a) has been aligned ; and

c) detecting a hybrid nucleic acid molecule formed between the polynucleotide in the biological sample and the aligned polynucleotide of step a).

44. A kit for detecting a polynucleotide of mycobacterial origin in a biological sample, comprising:

a) a substrate on which at least one polynucleotide contained in a recombinant BAC vector according to claim 23 or 29 has been aligned.

45. The method of claim 10, wherein the procedure by which the polynucleotide contained in a clone of a BAC DNA library is prepared, further comprises amplifying the polynucleotide insert.

46. The method of claim 10, wherein the procedure by which the polynucleotide contained in a clone of a BAC DNA library is prepared, further comprises digesting the polynucleotide insert with at least one restriction endonuclease.

47. The method of claim 45, further comprising digesting the amplified polynucleotide insert with at least one restriction endonuclease.

48. The Polynucleotide of claim 17, wherein the mycobacterium strain is *Mycobacterium tuberculosis*.

49. The method of claim 36, wherein the amplified mycobacterial DNA is detected by gel electrophoresis or with a labeled polynucleotide according to claim 11.

50. The kit of claim 40, further comprising a polynucleotide according to claim 11.

51. The kit of claim 42, further comprising reagents necessary to perform a hybridization reaction.

52. A method for physically mapping a polynucleotide of mycobacterial origin in a biological sample, said method comprising:

a) aligning at least one polynucleotide contained in a recombinant BAC vector according to claim 23 or 29 on the surface of a substrate;

b) contacting the polynucleotide in the biological sample with the substrate on which the polynucleotide of step a) has been aligned under hybridizing conditions; and

c) detecting the location of the hybridized polynucleotide from the biological sample.

53. The kit of claim 44, further comprising reagents necessary for labeling DNA and reagents necessary for performing a hybridization reaction.

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(57) Abstract

The present invention is directed to a method for isolating a polynucleotide of interest that is present or is expressed in a genome of a first mycobacterium strain and that is absent or altered in a genome of a second mycobacterium strain which is different from the first mycobacterium strain using a bacterial artificial chromosome (BAC) vector. The invention further relates to a polynucleotide isolated by this method and recombinant BAC vector used in this method. In addition the present invention comprises method and kit for detecting the presence of a mycobacteria in a biological sample.

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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PHILIPP W.J. ET AL.,: "Physical mapping of mycobacterium bovis BCG pasteur reveals differences from the genome map of mycobacterium tuberculosis H37Rv and from M. bovis" MICROBIOLOGY, vol. 142, - 1996 pages 3135-3145, XP002118720 cited in the application the whole document --- -/--	1-50

[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
14 October 1999	27/10/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Müller, F

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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